bio-protocol Preprint

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NMR measurements of phase-separating samples

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An abbreviated version of this protocol was published in Science Advances in Apr 2020

Measles virus nucleo- and phosphoproteins form liquid-like phase-separated compartments that promote nucleocapsid assembly

DOI: 10.1126/sciadv.aaz7095

Detailed protocol

Dear Requester
Below I attach the details of the NMR measurements of phase separating samples as described in the manuscript. These are standard experiments available on all commercial NMR spectrometers (in this case Bruker Avance III) and are very straightforward to set-up. I attach the exact pulse programs that we used that you can apply directly. Please let us know (martin.blackledge@ibs.fr) if any other parts of the protocol are not clear.

NMR measurements of phase separating samples

^{15}N labelled $P_{50}N_{525}$ and $P_{304\text{-}507}$ were premixed at different ratios and diluted in buffer so that final NaCl concentration was 150 mM and then loaded in 3 mm NMR tube. ^{1}H-^{15}N HSQCs and R_{1r} experiments were measured as described for non-phase separating samples. Pulsed field gradient NMR diffusion experiments were measured using ^{15}N-edited stimulated-echo (STE) diffusion experiments using a 300 ms diffusion delay at 16 to 20 gradient strengths ranging linearly from 0 to 0.55 T m^{-1} (*47*). ^{1}H-^{15}N HSQCs used for the development of the kinetic model were measured using both $P_{50}N_{525}$ and $P_{304\text{-}507}$ ^{15}N labeled samples at constant $P_{304\text{-}507}$ concentration of 75 μM and $P_{50}N_{525}$ 5%, 10%, 20% 50% 100%. The concentration of $P_{50}N_{525}$ and $P_{304\text{-}507}$ in the dilute phase was estimated on the basis of intensities of 23 for $P_{50}N_{525}$ and 9 for $P_{304\text{-}507}$ non-overlapping and non-interacting peaks which were summed and normalised by the intensities of the peaks in the spectra of the individual proteins.

Related files

Archive.zip

How to cite:(Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used)

1. Ruigrok, R. and Blackledge, M. (2021). NMR measurements of phase-separating samples. Bio-protocol Preprint. bio-protocol.org/prep990.
2. Guseva, S., Milles, S., Jensen, M. R., Salvi, N., Kleman, J., Maurin, D., Ruigrok, R. W. H. and Blackledge, M.(2020). Measles virus nucleo- and phosphoproteins form liquid-like phase-separated compartments that promote nucleocapsid assembly. Science Advances 6(14). DOI: 10.1126/sciadv.aaz7095

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